Patent
Attorney's Docket No. 025219-342

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	)	
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Marciacq, et al.	)	Group Art Unit: 1623
	)	
Application No.: 09/914,221	)	Examiner: Patrick T. Lewis
	)	
Filed: August 22, 2001	)	Confirmation No. 1827
	)	
For: PROCESS FOR MANUFACTURING MORPHOLINO-NUCLEOTIDES, AND USE THEREOF FOR THE ANALYSIS OF AND LABELLING OF NUCLEIC ACID SEQUENCES	)	

DECLARATION OF DIDIER MOLKO, D.Sc., Ph.D. UNDER 37 C.F.R. ∋ 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

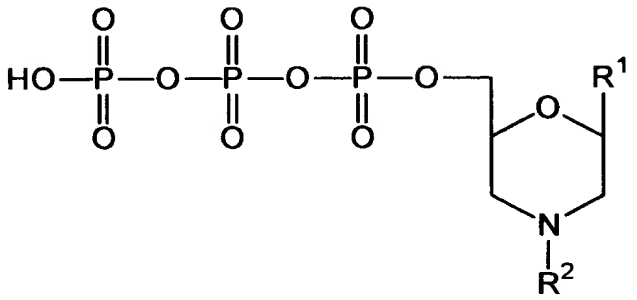
I, Didier Molko, D.Sc., Ph.D. hereby declare as follows:

1. I was a Director of CEA Grenoble Inorganic and Biological Chemistry Department, France until August 31, 2001.

2. I have 27 years of experience in the field of nucleotide chemistry. I am a co-author of 56 scientific publications and co-inventor of 8 patents. My curriculum vitae is attached hereto as Exhibit 1.

3. I am a co-inventor of the above-referenced application. I have reviewed the outstanding Office Action (paper No. 7) received for this application.

4. The presently claimed invention is directed to the use of nucleotide derivatives of formula (I)



(I)

in which R^1 represents a nucleic base and R^2 represents a group corresponding to one of the following formulae:

$-(CH_2)_n-NH_2$	$-(CH_2)_n-SH$
$-(CH_2)_n-COOH$	$-(CH_2)_n-OH$
$-(CH_2)_n-NH-R^3$	$-(CH_2)_n-SR^3$
$-(CH_2)_n-CO-R^3$	$-(CH_2)_n-OR^3$,

in which n is an integer ranging from 1 to 12 and R^3 is a group derived from a label, a protein, an enzyme, a fatty acid or a peptide.

5. In the nucleotide derivatives of formula (I) of the present invention, the substituted morpholine, which replaces the conventional saccharide portion, further comprises:

1) a hydroxymethyl function close to the ring oxygen, esterified with a triphosphoric acid group. This portion of the molecule mimics the 4',5' portion of nucleotides and allows binding by the polymerase or the transcriptase to the growing DNA or RNA chain.

2) an amine function substituted with R^2, which can optionally allow the grafting of a chromophore or of a biologically active group and, especially, which prevents the attachment of another nucleotide (interruption of the polymerization).

6. According to the present invention, R^3 may be selected from a very large set of well-known in the art and commercially available nucleotide labeling molecules. These molecules may be selected, for example, from radioactive products, luminescent, electroluminescent and fluorescent products, molecules capable of coupling with other molecules, molecules allowing interaction of antigen-antibody type, and enzymatic labels including hydrolases, particularly, phosphatases, esterases, ureases and glycosidases, or oxidoreductases, particularly, peroxidases.

7. Preparation of the compounds of formula (I) of the present invention wherein R^3 is a group derived from a label, a protein, an enzyme, a fatty acid or a peptide is based on the well-known methods as evidenced by the cited references [4]-[8] at page 11, line 1 through page 12, line 9 of the specification.

8. In our research, we have used two general procedures for synthesis of R^3 labeled morpholinonucleotides of formula (I).

9. The first method is described in the above-mentioned reference [7], patent FR-A- 2 710 068 –B1, issued on October 20, 1995, of which I am a co-inventor, and U.S. counterpart of which is U.S. Patent No. 5,721,341, issued on February 24, 1998. Our patents teach the two-phase method which consists of condensing the diamine (or amino acid) first and then grafting R^3 at the end of the chain wherein R^3 represents a protein containing a free amino site, an aminoalkyl polysterene or silica grafted with an alkyl amine chain.

10. The second method, which is utilized in the present application, comprises condensation of a compound of formula R_2-NH_2 (or R_3-$(CH_2)_n$-NH_2) with the opening product of the 2', 3' diol of the initial triphosphate nucleoside ribose cycle.

11. Our fluorescent morpholinonucleotide synthesis method uses <u>two very well-</u>

established textbook organic chemistry reactions which are well-known to those skilled in the art. See, for example, reference [4] cited in the specification.

- The first reaction consists of oxidative splitting of a vicinal diol with periodate ion resulting, when a triphosphate nucleoside ribose cycle is used, in dialdehyde formation.

- The second reaction is also a textbook reaction which includes the formation of a Schiff base adduct from an aldehyde and an amine, which is (R_3-$(CH_2)_n$-NH_2) in our case.

For the latter reaction, in our specific case, the primary amine is condensed on a dialdehyde, resulting in cyclization in the form of morpholine.

12. In our method, R^3 resides at the end of an alkylated chain while the reaction site, i.e. the primary amine, is located at the other end of the chain, separated from R^3 by one or more carbon atoms. It is highly improbable that the nature of R^3, which is not involved with the reaction site, would have any impact on the outcome of the reactions of amine condensation with aldehyde functions and/or cyclization.

13. Our description of the reaction with fluorescein demonstrates that the nature of R^3 is not essential for the purposes of the present invention. Our findings that fluorescein being a large substituent does not affect the outcome of the reaction, support my conclusion that for the purposes of the present invention, R^3 may be selected from a very large set of well-known in the art and commercially available nucleotide labeling molecules.

14. The disclosure of the specification in combination with the cited reference could only lead to the conclusion that the presently claimed invention is enabled.

15. It is my belief, as an expert in the field, that using the description of the present application, those of skill in the art would be able to and have, in fact, been able to make and use the claimed invention.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that all statements made herein are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Respectfully submitted,

By: _____
Didier Molko, D.Sc., Ph.D.

Date: May 12, 2003